RayBio® Human Fibronectin ELISA Kit

Catalog #: ELH-FN1

User Manual
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Caution:
Extraordinarily useful information enclosed

RayBiotech
The protein array pioneer

ISO 13485 Certified

3607 Parkway Lane, Suite 100
Norcross, GA 30092
Tel: 1-888-494-8555 (Toll Free) or 770-729-2992, Fax:770-206-2393
Web: www.RayBiotech.com, Email: info@raybiotech.com

RayBiotech, Inc.

RayBio® Human Fibronectin ELISA Kit Protocol

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Please read the entire manual carefully before starting your experiment

I. INTRODUCTION

The RayBio® Human Fibronectin ELISA kit is an in-vitro enzyme-linked immunosorbent assay for the quantitative measurement of human Fibronectin in serum, plasma and cell culture supernatants. This assay employs an antibody specific for human Fibronectin coated on a 96-well plate. Standards and samples are pipetted into the wells and Fibronectin present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human Fibronectin antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of Fibronectin bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

II. STORAGE

The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. For prepared reagent storage, see table below.

III. REAGENTS

Component	Size / Description	Storage / Stability After Preparation
Fibronectin Microplate (Item A)	96 wells (12 strips x 8 wells) coated with anti-Human Fibronectin.	1 month at 4°C*
Wash Buffer Concentrate (20X) (Item B)	25 ml of 20X concentrated solution.	1 month at 4°C
Standard Protein (Item C)	2 vials of Human Fibronectin. 1 vial is enough to run each standard in duplicate.	1 week at -80°C
Detection Antibody Fibronectin (Item F)	2 vials of biotinylated anti-Human Fibronectin. Each vial is enough to assay half the microplate.	5 days at 4°C
HRP-Streptavidin Concentrate (Item G)	200 µl 1,300X concentrated HRP-conjugated streptavidin.	Do not store and reuse.
TMB One-Step Substrate Reagent (Item H)	12 ml of 3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution.	N/A
Stop Solution (Item I)	8 ml of 0.2 M sulfuric acid.	N/A
Assay Diluent (Item E2)	15 ml of 5X concentrated buffer.	1 month at 4°C

*Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

IV. ADDITIONAL MATERIALS REQUIRED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µl to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. Log-log graph paper or computer and software for ELISA data analysis.
8. Tubes to prepare standard or sample dilutions.

V. REAGENT PREPARATION

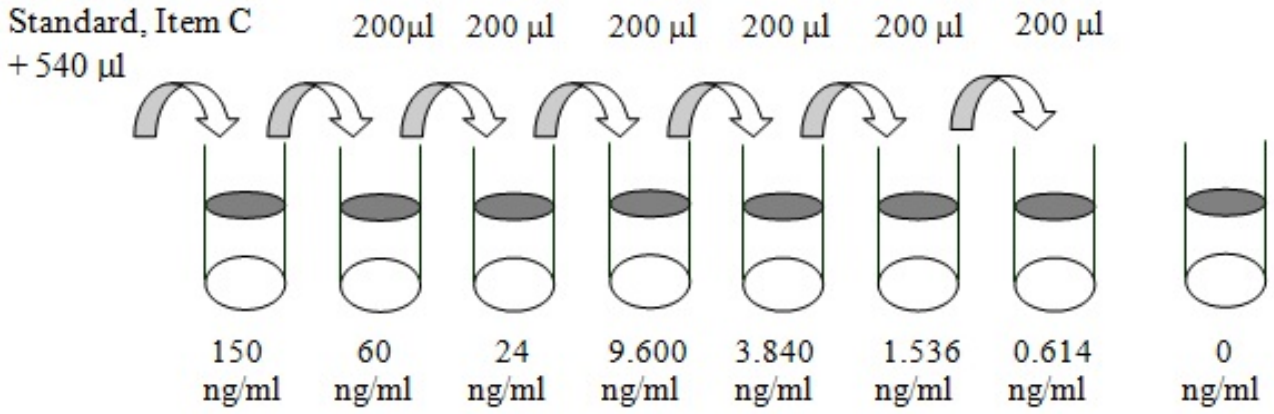
1. Bring all reagents and samples to room temperature (18 - 25ºC) before use.

2. Assay Diluent (Item E2) should be diluted 5-fold with deionized or distilled water before use.

3. Sample dilution: 1X Assay Diluent (Item E2) should be used for dilution of serum, plasma, and cell culture supernatant samples. The suggested dilution for normal serum/plasma is 10,000 fold.

   Note: Levels of Fibronectin may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

4. Preparation of standard: Briefly spin a vial of Item C. Add 540 µl of 1X Assay Diluent (Item E2; Assay Diluent should be diluted 5-fold with deionized or distilled water before use) into Item C vial to prepare a 150 ng/ml standard. Dissolve the powder thoroughly by a gentle mix. Pipette 300 µl of 1X Assay Diluent into each tube. Use the stock standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1X Assay Diluent serves as the zero standard (0 ng/ml).



5. If the Wash Concentrate (20X) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Buffer.

6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1X Assay Diluent (Item E2) into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4ºC for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent (Item E2) and used in step 5 of Part VI Assay Procedure.

7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 1,300-fold with 1X Assay Diluent (Item E2).

   For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 10 µl of HRP-Streptavidin concentrate into a tube with 13 ml 1X Assay Diluent to prepare a 1,300-fold diluted HRP-Streptavidin solution (do not store the diluted solution for next day use). Mix well.

VI. ASSAY PROCEDURE

1. Bring all reagents and samples to room temperature (18 - 25ºC) before use. It is recommended that all standards and samples be run at least in duplicate.

2. Label removable 8-well strips as appropriate for your experiment.

3. Add 100 µl of each standard (see Reagent Preparation step 3) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4ºC with gentle shaking.

4. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

5. Add 100 µl of 1X prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.

6. Discard the solution. Repeat the wash as in step 4.

7. Add 100 µl of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

8. Discard the solution. Repeat the wash as in step 4.

9. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

10. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

VII. ASSAY PROCEDURE SUMMARY

1. Prepare all reagents, samples and standards as instructed.

2. Add 100 µl standard or sample to each well. Incubate 2.5 hours at room temperature or over night at 4ºC.

3. Add 100 µl prepared biotin antibody to each well. Incubate 1 hour at room temperature.

4. Add 100 µl prepared Streptavidin solution. Incubate 45 minutes at room temperature.

5. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.

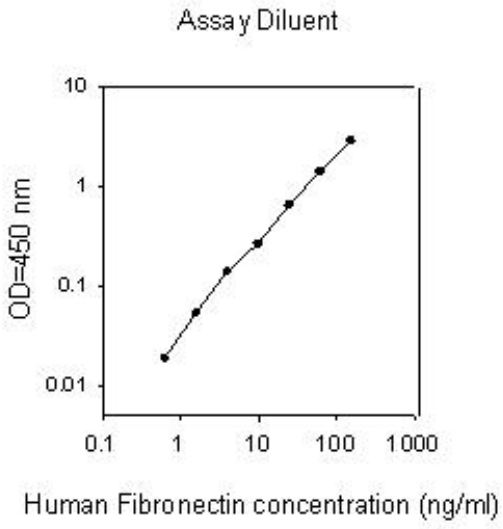
6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

VIII. CALCULATION OF RESULTS

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

A. TYPICAL DATA

These standard curves are for demonstration only. A standard curve must be run with each assay.



B. SENSITIVITY

The minimum detectable dose of Human Fibronectin was determined to be 0.6 ng/ml.

Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer).

C. SPIKING & RECOVERY

Recovery was determined by spiking various levels of Human php echo Fibronectin into the sample types listed below. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	92.94	80-105
Plasma	82.21	67-93
Cell culture media	71.95	68-82

D. LINEARITY

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected	89.08	75.24	81.04
	Range (%)	83-94	68-83	76-86
1:4	Average % of Expected	72.77	70.74	82.89
	Range (%)	69-76	69-74	78-87

E. REPRODUCIBILITY

Intra-Assay CV%: <10%

Inter-Assay CV%: <12%

IX. SPECIFICITY

This ELISA antibody pair detects human Fibronectin. Other species not determined.

X. TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	• Inaccurate pipetting • Improper standard dilution	• Check pipettes • Briefly centrifuge Item C and dissolve the powder thoroughly by gently mixing
Low signal	• Improper preparation of standard and/or biotinylated antibody • Too brief incubation times • Inadequate reagent volumes or improper dilution	• Briefly spin down vials before opening. Dissolve the powder thoroughly. • Ensure sufficient incubation time; assay procedure step 2 may be done overnight • Check pipettes and ensure correct preparation
Large CV	• Inaccurate pipetting • Air bubbles in wells	• Check pipettes • Remove bubbles in wells
High background	• Plate is insufficiently washed • Contaminated wash buffer	• Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed. • Make fresh wash buffer
Low sensitivity	• Improper storage of the ELISA kit • Stop solution	• Store your standard at <-70ºC after reconstitution, others at 4ºC. Keep substrate solution protected from light. • Add stop solution to each well before reading plate

RayBio® ELISA Kits

Over 2,000 ELISA kits available, visit www.RayBiotech.com/ELISA-Kits.html for details.

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